

What is claimed is:

Sub B7
5 1. An isolated and purified superantigen toxin DNA fragment which has been altered such that binding of the encoded altered toxin to either the MHC class II or T cell antigen receptor is altered.

2. An isolated and purified DNA fragment according to claim 1, wherein said superantigen toxin is Staphylococcal enterotoxin A having the sequence of
10 SEQ ID NO:1 or a portion thereof, or an allelic portion thereof.

3. An isolated and purified DNA fragment according to claim 1, wherein said superantigen toxin
15 is Staphylococcal enterotoxin A having the sequence of SEQ ID NO:3 or a portion thereof, or an allelic portion thereof.

20 4. An isolated and purified DNA fragment according to claim 1, wherein said superantigen toxin is Staphylococcal enterotoxin B having the sequence of
Sub C3
SEQ ID NO:5 or a portion thereof, or an allelic portion thereof.

25 5. An isolated and purified DNA fragment according to claim 1, wherein said superantigen toxin is Staphylococcal enterotoxin B having the sequence of SEQ ID NO:7 or a portion thereof, or an allelic portion thereof.

30 6. An isolated and purified DNA fragment according to claim 1, wherein said superantigen toxin is Staphylococcal enterotoxin B having the sequence of
Sub C4
35 SEQ ID NO:9 or a portion thereof, or an allelic portion thereof.

5

10

15

20

25

30

Sub B1

SUB
C5

5

13. An isolated and purified DNA fragment according to claim 5, wherein said fragment encodes the amino acid sequence of SEQ ID NO:8 or a portion thereof, or an allelic portion thereof.

10

14. An isolated and purified DNA fragment according to claim 6, wherein said fragment encodes the amino acid sequence of SEQ ID NO:10 or a portion thereof, or an allelic portion thereof.

15

15. An isolated and purified DNA fragment according to claim 7, wherein said fragment encodes the amino acid sequence of SEQ ID NO:12 or a portion thereof, or an allelic portion thereof.

20

16. An isolated and purified DNA fragment according to claim 8, wherein said fragment encodes the amino acid sequence of SEQ ID NO:14 or a portion thereof, or an allelic portion thereof.

25

17. An isolated and purified DNA fragment according to claim 9, wherein said fragment encodes the amino acid sequence of SEQ ID NO:16 or a portion thereof, or an allelic portion thereof.

SUB
C6

30

18. A recombinant DNA construct comprising:
(i) a vector, and
(ii) an isolated and purified altered superantigen toxin DNA fragment according to claim 1.

19. A recombinant DNA construct according to claim 18, wherein said DNA fragment has the sequence according to SEQ ID NO:1 or a portion thereof, or an allelic portion thereof.

20. A recombinant DNA construct according to claim 18, wherein said DNA fragment has the sequence according to SEQ ID NO:3 or a portion thereof, or an allelic portion thereof. *E*

SUB C7
21. A recombinant DNA construct according to claim 18, wherein said DNA fragment has the sequence according to SEQ ID NO:5 or a portion thereof, or an allelic portion thereof.

22. A recombinant DNA construct according to claim 18, wherein said DNA fragment has the sequence according to SEQ ID NO:7 or a portion thereof, or an allelic portion thereof.

23. A recombinant DNA construct according to claim 18, wherein said DNA fragment has the sequence according to SEQ ID NO:9 or a portion thereof, or an allelic portion thereof.

24. A recombinant DNA construct according to claim 18, wherein said DNA fragment has the sequence according to SEQ ID NO:11 or a portion thereof, or an allelic portion thereof.

25. A recombinant DNA construct according to claim 18, wherein said DNA fragment has the sequence according to SEQ ID NO:13 or a portion thereof, or an allelic portion thereof. *E*

26. A recombinant DNA construct according to claim 18, wherein said DNA fragment has the sequence according to SEQ ID NO:15 or a portion thereof, or an allelic portion thereof.

5

28. The recombinant DNA construct according to claim 20, wherein said DNA fragment encodes the amino acids sequence specified in SEQ ID NO:4.

10

29. The recombinant DNA construct according to claim 21, wherein said DNA fragment encodes the amino acids sequence specified in SEQ ID NO:6.

15

30. The recombinant DNA construct according to claim ~~22~~¹⁸, wherein said DNA fragment encodes the amino acids sequence specified in SEQ ID NO:8.

20

31. The recombinant DNA construct according to claim 23, wherein said DNA fragment encodes the amino acids sequence specified in SEQ ID NO:10.

25

32. The recombinant DNA construct according to claim 24, wherein said DNA fragment encodes the amino acids sequence specified in SEQ ID NO:12.

30

33. The recombinant DNA construct according to claim 25, wherein said DNA fragment encodes the amino acids sequence specified in SEQ ID NO:14.

34. The recombinant DNA construct according to claim 26, wherein said DNA fragment encodes the amino acids sequence specified in SEQ ID NO:16.

35

35. A recombinant DNA construct according to claim 19 wherein said construct is pETA489270P.

10/88

5

10

10

15

claim 25 wherein said ~~construct~~ is pETSEC45.

20

25

SUB C8 >

30

recombinant DNA construct according to claim 28.

sub
ca

48. A host cell transformed with a
5 recombinant DNA construct according to claim 30.

10 50. A host cell transformed with a
recombinant DNA construct according to claim 32.

~~52. A host cell transformed with a recombinant DNA construct according to claim 34.~~

54. A host cell according to claim 45,
wherein said cell is prokaryotic.

56. A host cell according to claim 47,
wherein said cell is prokaryotic.

58. A host cell according to claim 49,
35 wherein said cell is prokaryotic.

59. A host cell according to claim 50,
wherein said cell is prokaryotic.

5 60. A host cell according to claim 51,
wherein said cell is prokaryotic.

61. A host cell ^ε according to claim 52,
wherein said cell is prokaryotic.

10 62. A method for producing altered
superantigen toxin comprising culturing the cells
according to claim 44, under conditions such that said
DNA fragment is expressed and said superantigen toxin
15 is thereby produced, and isolating said superantigen
toxin.

20 63. A method for producing altered
superantigen toxin comprising culturing the cells
according to claim 45, under conditions such that said
DNA fragment is expressed and said superantigen toxin
is thereby produced, and isolating said superantigen
toxin.

25 64. A method ^ε for producing altered
superantigen toxin comprising culturing the cells
according to claim 46, under conditions such that said
DNA fragment is expressed and said superantigen toxin
is thereby produced, and isolating said superantigen
30 toxin.

65. A method for producing altered
superantigen toxin comprising culturing the cells
according to claim 47, under conditions such that said
35 DNA fragment is expressed and said superantigen toxin

is thereby produced, and isolating said superantigen toxin.

5 66. A method for producing altered superantigen toxin comprising culturing the cells according to claim 48, under conditions such that said DNA fragment is expressed and said superantigen toxin is thereby produced, and isolating said superantigen toxin.

10 67. A method for producing altered superantigen toxin comprising culturing the cells according to claim 49, under conditions such that said DNA fragment is expressed and said superantigen toxin is thereby produced, and isolating said superantigen toxin.

20 68. A method for producing altered superantigen toxin comprising culturing the cells according to claim 50, under conditions such that said DNA fragment is expressed and said superantigen toxin is thereby produced, and isolating said superantigen toxin.

25 69. A method for producing altered superantigen toxin comprising culturing the cells according to claim 51, under conditions such that said DNA fragment is expressed and said superantigen toxin is thereby produced, and isolating said superantigen toxin. E

30 70. A method for producing altered superantigen toxin comprising culturing the cells according to claim 52, under conditions such that said DNA fragment is expressed and said superantigen toxin

is thereby produced, and isolating said superantigen toxin.

5 71. An isolated and purified superantigen toxin which has been altered such that binding of the encoded altered toxin to either the MHC class II or T cell antigen receptor is altered.

10 72. An isolated and purified superantigen toxin according to claim 71 wherein said toxin is staphylococcal enterotoxin A.

15 73. An isolated and purified superantigen toxin according to claim 71 wherein said toxin is staphylococcal enterotoxin B.

20 74. An isolated and purified superantigen toxin according to claim 71 wherein said toxin is staphylococcal toxin shock syndrome toxin-1.

25 75. An isolated and purified superantigen toxin according to claim 71 wherein said toxin is staphylococcal enterotoxin C1.

30 76. An altered SEA superantigen toxin peptide according to claim 72 wherein position 92 has been changed to alanine.

35 77. An altered SEA superantigen toxin peptide according to claim 72 wherein position 70 has been changed to arginine.

78. An altered SEA superantigen toxin peptide according to claim 72 wherein position 48 has been changed to arginine.

79. An altered SEA superantigen toxin peptide according to claim 72 wherein position 64 has been mutated to alanine.

5

80. An altered SEB superantigen toxin peptide according to claim 73 wherein position 115 has been changed to alanine.

10

81. An altered SEB superantigen toxin peptide according to claim 73 wherein position 89 has been changed to alanine.

15

82. An altered SEB superantigen toxin peptide according to claim 73 wherein position 67 has been changed to glutamine.

20

83. An altered SEB superantigen toxin peptide according to claim 73 wherein position 94 has been changed to alanine.

25

84. An altered SEB superantigen toxin peptide according to claim 73 wherein position 61 has been changed to alanine.

85. A method for the diagnosis of superantigen-associated bacterial infection comprising the steps of:

30

(i) contacting a sample from an individual suspected of having a superantigen-associated bacterial infection with altered superantigen toxin; and

35

(ii) detecting the presence or absence of a superantigen-associated bacterial infection by detecting the presence or absence of a complex formed

between the altered superantigen toxin and antibodies specific therefor in the sample.

5 86. A method for the diagnosis of a superantigen toxin-associated bacterial infection according to claim 63 wherein the altered superantigen toxin is chosen from the group consisting of SPEa, SEB, SEA, TSST-1, SEC-1.

10 87. A superantigen toxin-associated infection diagnostic kit comprising an altered superantigen toxin according to claim 58 wherein said toxin is chosen from the group consisting of SPEa, SEB, SEA, TSST-1, and SEC-1, and ancillary reagents
15 suitable for use in detecting the presence or absence of antibodies against superantigen toxin in a mammalian sample.

20 88. A vaccine comprising an altered superantigen toxin according to claim 58 effective for the production of antigenic and immunogenic response resulting in the protection of a mammal against superantigen-associated bacterial infection.

25 89. A vaccine according to claim 66 wherein said altered superantigen toxin is chosen from the group consisting of SPEa, SEB, SEA, TSST-1, and SEC-1.

30 90. A vaccine according to claim 67 wherein said vaccine further comprises at least one other different altered superantigen toxin chosen from the group consisting of SPEa, SEB, SEA, TSST-1, and SEC-1.

91. A vaccine according to claim 66, wherein the superantigen toxin is SEB and the vaccine is identified as B899445.

5 92. A vaccine according to claim 66, wherein the superantigen toxin is SEA and the vaccine is identified as A489270.

10 93. A bivalent vaccine according to claim 68 wherein said altered superantigen toxins are SEA and SEB.

15 94. A bivalent vaccine according to claim 71 wherein said toxin SEA is A489270 and SEB is B899445.

20 95. A multivalent vaccine against superantigen-associated bacterial infections comprising a combination of altered superantigen toxins selected from the group consisting essentially of TSST-1, SPEa, SEA, SEB, and SEC-1 or any portion or allelic form thereof, capable of eliciting protective antibodies against superantigen toxins in a pharmaceutically acceptable excipient in a pharmaceutically acceptable amount.

25 96. A therapeutic method for the treatment or amelioration of a superantigen-associated bacterial infection said method comprising administering to an individual in need of such treatment an effective amount of sera from individuals immunized with one of more altered superantigen toxin vaccine according to claim 67 in a pharmaceutically acceptable dose in a pharmaceutically acceptable excipient.

30

97. A therapeutic method for the treatment or amelioration of a superantigen-associated bacterial infection, said method comprising administering to an individual in need of such treatment an effective
5 amount of antibodies against altered superantigen toxins in a pharmaceutically acceptable dose in a pharmaceutically acceptable excipient.

98. A therapeutic method for the treatment
10 or amelioration of a superantigen-associated bacterial infection, said method comprising administering to an individual in need of such treatment an effective amount of altered superantigen toxins from streptococcal and staphylococcal bacteria in order to
15 inhibit adhesion of superantigen bacterial toxin to MHC class II or T cell receptors by competitive inhibition of these interactions in a pharmaceutically acceptable dose in a pharmaceutically acceptable excipient.

99. A therapeutic method for the treatment
20 of diseases that may not be associated directly with superantigen toxins by causing specific nonresponsiveness of T cell subsets or by expanding or stimulating specific T cell subsets, in vivo or ex
25 vivo by use of altered superantigen toxin.

30

Add A1

Add Gb

Add IZ